INTERNATIONAL JOURNAL OF INNOVATIVE RESEARCH & DEVELOPMENT

ISSN 2278 – 0211 (Online)

Effects of Feminine Wash (Soap) on Some Pathogenic Bacteria Causing Urinary Tract Infections (UTIS)

Aliyu M. S.
Lecturer, Department of Microbiology, Ahmadu Bello University, Zaria, Nigeria
Musa B.
Graduate Assistant, Department of Microbiology, Ahmadu Bello University, Zaria, Nigeria
Hussaini I. M.
Postgraduate Student, Department of Microbiology, Ahmadu Bello University, Zaria, Nigeria
Charanchi A. S.
Postgraduate Student, Department of Microbiology, Ahmadu Bello University, Zaria, Nigeria

Abstract:
The use of feminine wash (soap) is of wide distribution among women of fertile ages for the relief of the symptoms of vaginal infections which includes: Itching, burning sensation, vaginal discharge, embarrassing odor among others. Vaginal infection which could either be caused by yeast (Thrush), bacteria (Bacterial vaginosis), and/ or even parasites (Vaginalis), is a frequent and common distressing disease affecting up to 10-75% of women of fertile ages most of which have had recurrent episodes. This study was aimed to determine the effects of feminine washes (soaps) on Staphylococcus aureus, Candida albicans, and Escherichia coli. Two hundred swab samples were collected using sterile swab sticks and transported to the laboratory for processing. The samples were inoculated each onto potato dextrose agar (PDA), Mannitol Salt Agar (MSA) and Chocolate agar. The plates were then incubated at 37oC overnight, following which the isolates were identified by Gram staining and various biochemical tests. The identified isolates were then standardized by comparing with the turbidity of 0.5 McFarland standard, for the susceptibility test using agar well diffusion technique. Four different dilutions each of three different feminine washes labelled A, B and C, were prepared by serial dilution of each stock sample (100%) using half-fold dilution to obtain 50%, 25%, 12% and 6.25% concentrations. The feminine wash labelled A was found to be the most effective at all concentrations against S. aureus and E. coli but C. albicans was only susceptible at 100% and 50% concentrations. All the three isolates were found to be resistant to the feminine wash labelled C. It can therefore be concluded that, the feminine wash A is the best soap for preventing and/or managing vaginal infections.

Keywords: *Feminine wash, Pathogenic Bacteria, Urinary Tract Infections*

1. Introduction

The use of feminine wash (soap) is of wide distribution among women of fertile ages for the relief of the symptoms of vaginal infections which includes: Itching, burning sensation, vaginal discharge, embarrassing odour among others (Mayo Clinic, 2009). Vaginal infection which could either be caused by yeast (thrush), Bacteria (Bacterial vaginosis), and or even parasites (vaginalis), is a frequent and common distressing disease affecting up to 10-75% of women of fertile ages most of which have had recurrent episodes (Pietrella *et al.*, 2011) and might be due to the following reasons.

A healthy vagina is colonized by mutually symbiotic microorganisms also known as normal flora, or microbiota, which include; *Lactobacillus acidophilus*, *Lactobacillus fermentum*, and *Candida albicans* at a suppressed growth. These microbiota of the vagina normally provide a natural antimicrobial activity similar to that of hydrogen peroxide by secreting enzymes that ferment glycogen produced by the vaginal epithelium to lactic acid which gives a healthy vagina a pH of 4.4 to 4.6 and this acidic nature of the vagina retards the growth of many strains of pathogenic microbes (Prescott *et al.,* 2008).

However, any attempt to upset the balance of the vaginal environment can favour the pathogenic microbes and also overgrowth of its own normal flora such as *Candida albicans* to invade and cause vaginal infection (Pietrella *et al.*, 2011).

Interestingly, antibiotics are used in the treatment of some ailments other than vaginal infection; such ailments can be typhoid fever, skin disease, wound infection; septic and aseptic pneumonia etc. However, the high doses of antibiotics used, often suppress the growth of microorganism in the body system including the beneficial ones such as the normal microbiota of the vagina thereby altering the balance of the vaginal environment as a result.

2. Materials and Methodology

2.1. Study Population
The population comprises of patients with complaints of vaginal infection visiting sick bay Ahmadu Bello University, Zaria and Ahmadu Bello University, Teaching Hospital.

2.2. Sample Size
Two hundred samples were collected and analyzed.

2.3. Sample Collection
A sterile speculum was inserted into the vagina to hold the vagina open. A sterile swab stick was then used to collect samples by rubbing the swab stick on the vaginal walls. The speculum was then removed and the swab transported to the laboratory for analysis (Cheesbrough, 2000).

2.4. Isolation

2.4.1. Culture
The samples collected were each inoculated onto a Potato Dextrose Agar (PDA), Mannitol Salt Agar (MSA) and Chocolate agar plates and these were incubated for 24 hours at 37^{o}C (Prescott et al., 2008).

2.5. Morphological Characterization

2.5.1. Gram Staining
After 24 hours of incubation, isolates obtained were then Gram-stained by picking a distinct colony and smeared on a free clean glass slide with a drop of distilled water, this was then allowed to air dry. The smear was then fixed by passing the slide over a Bunsen flame for three quick successions in order to adequately preserve the overall morphology but not the structures within the cells (Prescott *et al.,* 2008). The fixed smear was then stained with the basic dye crystal violet for 60 seconds, washed and then flooded with Gram's iodine for also 60 seconds which function is to increase the interaction between the peptidoglycan and the crystal violet dye so that the cell is stained more strongly. The smear was next decolorized by washing with acetone and lastly the smear was counter-stained with safranin. The cell morphology such as the cell shape, colour and arrangement were observed and recorded (Cheesbrough, 2000). After Gram staining, the isolates were separately preserved on appropriately labelled nutrient agar slants and stored in refrigerator at 8^{o}C for further analyses.

2.6. Biochemical Characterization

2.6.1. Gram Positive Cocci
Typical Gram positive cocci that appeared in singly, in pairs or in clusters, were screened for coagulase and catalase enzyme production so as to identify *Staphylococcus aureus*. The coagulase test remains the most popular test for the identification of *S. aureus* in routine work. It is also used to distinguish between *S. aureus* and other members of the genus *Staphylococcus* such as *S. epidermidis*.

2.6.2. Procedure
The catalase test is used in differentiating members of the genus *Staphylococcus* and *Streptococcus* species. This a test was done by placing a drop of 3% hydrogen peroxide on a clean glass slide, a bit of the isolate was then picked with a sterilized wire loop and emulsified in the hydrogen peroxide. Bubbling and frothing indicated a positive test for *Staphylococcus* (Cheesbrough, 2000).
Coagulase test was carried out by placing drop of physiological saline on a clean glass slide and one colony was emulsified, after which a loopful of human plasma was then added to the bacterial suspension and mixed carefully, the slide was then rocked by tilting back and forth for 1 minute. Appearance of clumped cells or precipitation indicated that the bacterium was coagulase positive (Fawole *et al*., 1988).

2.6.3. Gram Negative Bacilli
Various tests were carried out to identify *Escherichia coli* which include the following:

2.6.3.1. Indole Test
The bit of the isolate was inoculated into peptone water and incubated for 24 hours after which 3-drops of Kovac's indole reagent was added and shaken gently, the appearance of a red colour ring above the broth indicated a positive result (Cheesbrough, 2000).
2.6.3.2. Methyl red (MR) and Voges Proskauer (VP) Tests
A small pinch of the isolate was inoculated into test tube containing MR-VP broth, in duplicate and incubated for 48-72 hours. A methyl red reagent was added into one of the tube and Voges-Proskauer reagents (5 drops of potassium hydroxide and 15 drops of α -

naphthol) were added into the second tube containing same isolate. Appearance of a red colored ring at the broth-air interface indicated positive result for both MR and VP tests while a yellow colored ring indicated negative result for methyl red test and cloudy colouration indicates negative result for V.P (Prescott *et. al.,* 2008).

2.6.3.3. Sugar Fermentation Tests on Triple Sugar Iron Agar
A straight wire was used to obtain isolates and inoculated on a triple sugar iron agar slant by stabbing the butt to two-third of the entire length and streaking the surface of the slant. The slants were then capped loosely and incubated for 24hours. Formation of yellow colouration of butt and slant (which insinuate glucose and lactose or sucrose fermentation) with gas production (A/AG) indicated a complete positive test for *E. coli* (Prescott *et al.,* 2008).

2.6.3.4. Citrate Utilization Test
A Simmon's citrate agar slant in bijou bottles were inoculated with the isolate and incubated for 24 hours. The appearance of a deep blue colour indicated a positive reaction and a green colour (agar colour) indicated negative reaction *(*Prescott *et al.,* 2008*).*

2.7. Screening of Feminine Wash (Soaps) for Antimicrobial Activity

2.7.1. Agar-well Diffusion Method
The susceptibilities of the test organism were assayed using agar well diffusion method as described by Ndukwe *et al*. (2005).
Three different feminine washes named Dr James feminine wash, summer eve feminine wash and Fem tight and V. wash were purchased. Different concentrations; 100%, 50%, 25%, 12.5% and 6.25% volume by volume for the liquid soap and same concentration in weight by volume for the solid soaps were prepared using sterile distilled water following serial dilution: For the liquid soaps, 4mls of the soaps (Dr James and Summer eve feminine wash) were each aseptically poured into a sterile test tube as the 100% concentration (stock). Four other sterile test tubes each containing 2mls of distilled water were placed next to the stock; 2mls of the stock was transferred into the second test tube to obtain 50% concentration. The dilution continued until a concentration of 6.25% was achieved (Lenette *et al.,* 1975).
Same technique was used for the solid soap except for the preparation of the stock; 2grams of the soap was pounded using a sterile mortar and pestle and dissolved in 2mls of distilled water to make the 100% concentration and subsequent concentration were prepared following same technique (Cheesbrough, 2008).
Standardization of the inoculums (isolate) was prepared using McFarland standards of scale 0.5. On a Mueller Hinton Agar, 0.1mls of each the standardized inoculums *(Staphylococcus aureus, Escherichia coli and Candida* albicans) was spread uniformly and five Punch holes were made to accommodate different dilutions (concentrations) of the soaps; A total of six plates; three each for test and control were labelled for each feminine wash.
Different dilutions of the 3 Feminine washes were then introduced into the wells starting with the lowest dilution. On the control plates, for *Staphylococcus* and *E. coli*, a standard disk (Ciprofloxacin) was introduced to serve as control. Plates were then incubated at 37^{o}c and zones of inhibition where produced, were measured in millimeter after 24hr of incubation.

3. Results
The number of the selected species encountered in the 200 vaginal swab samples collected from patients with vaginal infections attending sickbay A.B.U Zaria and A.B.U Teaching Hospital from June – September 2011 were represented in table one. The selected organisms in the order of decreasing occurrence in the survey include *Candida albicans, Staphylococcus aureus*, and *Escherichia coli.* More so the number of isolates, percentage prevalence and the susceptibility pattern of the three organisms to the three soaps used at various concentrations were also represented in Tables below.

Organisms	Number of isolates	Prevalence (%)
E. coli	27	20.30
S. aureus	46	34.59
C. albicans	60	45.11
Total	133	100

Table 1: Prevalence of Isolates from Samples

Table 1 shows the number of the three isolates with significant growth and the percentage prevalence of the three organisms with *Candida albicans* having the highest prevalence (45.11%) followed by *Staphylococcus aureus* (34.59%) and then *Escherichia coli* (20.30).

Table 2 shows the susceptibility pattern of the three isolates to Dr. James feminine wash with *Escherichia coli* being more susceptible to the soap at lower concentrations of 6.25%, 12.5% and 25% followed by *Staphylococcus aureus* at same concentration. But *Candida albicans* was resistance to the soap at these lower concentrations (5mm, diameter of the cork borer).
At 50% and 100% however, *S. aureus* was the most susceptible followed by *E. coli* and then *C. albicans.*

Isolates	Concentrations (%)/Zones of inhibition (mm)					control
	6.25	12.5	25	50	100	
E. coli	25	27	30	34	35	35
S. aureus	15	23	24	40	50	35
C. albicans	5	5	5	16	18	

Table 2: Susceptibility Patterns of Isolates to Dr. James Feminine Wash.

Table 3 shows the susceptibility patterns of the three isolates to eve feminine wash soap and was found that the soap had effect on *E. coli* only at 50% and 100% and on *C. albican* only at 100% concentration. Isolates resistant to the soap at one concentration or the other were given the diameter of the cork borer.

Isolates	Concentrations (%)/ Diameter of zone of inhibition Control					
	6.25	12.5	25	50	100	
E. coli	5	5	5	17	25	28
S.aureus	5	5	5	5	5	35
C.albicans	5	5	5	5	23	

Table 3: Sensitivity Pattern of Isolates to Eve Feminine Wash.

Table 4 shows the susceptibility patterns of the three isolates to V.Wash feminine soap and it was found that the three isolates were resistant to the soap at all concentrations carries the Diameter of the cork borer(5mm).

	Concentrations (%)	/zones	of	Inhibition	(mm)	
Isolates	6.25	12.5	25	50	100	Control
E.coli	5	5	5	5	5	30
S.aureus	5	5	5	5	5	18
C.albicans	5	5	5	5	5	

Table 4: Sensitivity Patterns of Isolates to V. Wash

4. Discussion

Total numbers of 200 samples were collected and 133 yielded significant growth. Out of the significant isolates obtained *Candida albicans* was the most prevalent (45.11%), and might be due to the fact that *Candida albicans* is a normal flora of the vagina as well as an opportunistic pathogen, the second most prevalent pathogen is *Staphylococcus aureus* with the prevalence of 34.59% and might be due to the fact that S.*aureus* is a normal flora of the skin as such can easily contaminate the vagina. E.*coli* is the least prevalent organisms amongst the three isolate with a prevalence of 20.30%.

Dr. James feminine wash was the most effective soap amongst the three feminine wash (soaps). It had better effects on *S. aureus* at all concentrations. 100% and 50% concentration of this soap shows a diameter of zone of inhibition (50mm and 40mm respectively) that is greater than that of ciprofloxacin (30mm) that was used as control. It also had effect on *E. coli* at all concentration with equal diameter of inhibition for 100% concentration with that of the control. However, Dr. James was selective for *Candida albicans* as it had no effect to this organism at 6.25, 12.5 and 25% concentration. It showed effect at 50% and 100% concentration (16mm and 18mm diameter respectively).

This might be due to the fact that *Candida albicans* is a normal flora of the vagina at a suppressed level and pathogenic at a higher level as such the soap is reducing the population of this organism to a level that makes it a normal flora.

Summer Eve feminine wash is the next effective after Dr. James it had effect on *Escherichia coli* only at 50% and 100% concentration (17mm and 25mm respectively), and at 100% concentration affects *Candida albicans* (23mm diameter of zone of inhibition) and had no effect on *Staphylococcus aureus* at all concentrations.

Finally, Fem tight and antiseptic V-wash had no effect at all concentration on the three isolates (*Candida albicans*, *Escherichia* coli, *and Staphylococcus aureus*) so carries the diameter of the cork borer (5mm).

5. References

i. Anderson, M. R., Klink, K. and Cohrsen, A. (2004). Evaluation of vaginal complaints. Journal of American Medical Association. **291**(11):1368-79.

ii. Botash, A. S. (2005). Emergency-medicin Vaginitis:common causes,symptoms and treatment. 61747html

iii. Brooks, G. F., Caroll C.K, Butel, J. S. and Morse, S. A. (2001). Jawetz E, Melnick and Adelberg's Medical Microbiology,24th Edition, McGraw Hill Comp. Inc. p.292

iv. Bump, R. C. and Buesching, W. J. (1988). Bacterial vaginosis in virgins and sexually active adolescent females: evidence against exclusive sexual transmission. American Journal of Obstetrics and Gynecology.**158** (4):935-9.

v. Callahan, D. B., Weinberg, M. and Gunn, R. A. (2003). Bacterial vaginosis in Pregnancy: diagnosis and treatment practices of physicians in San Diego, California, 1999. Sexually Transmitted Diseases. Aug. ;30(8):645-9.

vi. Carr, P. L., Felsenstein, D. and Friedman, ft H. (1998). Evaluation and Management of Vaginitis. Journal of General International Medicine. **13**(5):335-46.

vii. Cheesbrough, M. (2000). District Laboratory Practice in Tropical Countries. Part 2. Cambridge University Press. pp.90 - 96.

viii. Coco, A. S. and Vanderbosche, M. (2000). Infectious vaginitis-an accurate diagnosis is essential and attainable. Postgraduate Medicine. **17**(4):63-74.

ix. Donder, G. G., Vareecken, A., Bosmans, E., Dekeersmaecker, A., Salembier, G. and Spitz, B. (2002). Definition of a type of abnormal vaginal flora that is distinct from bacterial vaginosis: aerobic vaginitis. British Journal of Obstetrics and Gynecology. January.;**109**(1):34-43.

x. Egan, M. E. and Lipsky, M.S. (2000). Diagnosis of Vaginitis. American Family Physician. 62:1095-1 04.

xi. Eltabbakh, G. H., Elabbakh, G. D., Broekhuizen, F. F., (1995). Value of wet mount and cervical cultures at the time of cervical cytology in asymptomatic women. Obstetrics and Gynecology. **85**(4):499-503.

xii. Emele, F. E., Anyiwo, C. E. and Fadunsi, A. A. (2004). Aetiologic profile of vaginal infections in okoto, Nigeria. Journal of Biomedical Investigations. **2**(2):57-62.

xiii. Fawole, M. O and Oso, B.A(1988) Laboratory manual of microbiology spectrum books limited, Ibadan, Nigeria pg 22-23.

xiv. Free Medical Dictionary Last Updated: Jun 30, 2009 - Vaginitis Definition

xv. Gale: Contemporary Women's Issues. Healthy Women, Dec. 2010.Yeast Infections Web. 7 Apr.2011

xvi. Haefner, H. K. (1999). Current evaluations and Management of vulvovaginitis. Clinical Obstetrics and Gynecology. **42**:184-95.

xvii. Hay, P. E. (1998). Recurrent bacterial vaginosis. Dermatology Clinical. **16**:769 (Suppl):65-9.

xviii. Hill, G. B. (1993). The Microbiology of bacterial vaginosis. American Journal of Obstetrics and Gynecology. **169**(2pt 2): 450-4.

xix. Hoist, E., Wathne, B., Horelius, B. and Mardh, P. A. (1987). Bacterial vaginosis:Microbiological and clinical findings. European Journal of Clinical Microbiology. **8**:536-541.

xx. Jaquiery A, Stylianopoulos A, Hogg G, Grover S (1999). Vulvovaginitis: clinical features, aetiology, and microbiology of the genital tract. Archives of diseases in childhood 81(1):64–7.doi:10.1136/adc.81.1.64.PMC 1717979.PMID 10373139. http://adc.bmj.com/cgi/pmidlookup?view=long&pmid=10373139